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DOCTORAL THESIS

BIOLOGICAL AND BIOCHEMICAL SYSTEMS OF DEGRADATION OF NATURAL LEATHER PRODUCTS

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SUMMARY

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The leather industry produces annually tonnes of waste that are considered to be extremely damaging to the environment. The solid remnants generated by the leather industry contain as a basic element the protein, but because of the substances used in the processing, they become very hard to decay, remaining as waste or incinerating producing also environmental pollution.

Nowadays the biotechnology sector can use this waste as a microbial substrate for enzyme production. The enzymes obtained may have plenty of uses and may cover various needs. The skin being a material based on organic carbon, rich in proteins can be used to improve soil in nitrogen and mineral substances, which can lead to a good growth and development of crops in organic farming.

The purpose of theresearch was to obtain an enzymatic preparation used for the degradation of natural abusers.

The research objectives were:

• Isolation of microorganisms with efficiency in accelerated degradation of waste from the leather industry

• Obtaining an hydrolysed product that could be used in organic farming as a bio fertilizer.

The experiments were conducted under laboratory conditions, in the Faculty of Biotechnologies, University of Agronomic Sciences and Veterinary Medicine Bucharest.

In order to carry out biodegradation experiments, several types of fur and leather with chromium from industrial sectors have been tested.

Pieces of leather and natural fur of sheep, tanned with chromium, as well as pieces of organic leather and fur, materials that were made available by the Bucharest Leather Institute, were taken into work.

For the screening of microorganisms, isolation and selection were taken into account by common microbiological methods.

The biological material used in the biodegradation of natural fur consisted of three bacterial strains that were isolated following the degradation of some scrap of fur and chromium tanned leather from the Bucharest Leather Institute.

The thesis is structured in two parts:

The first includes data from literature on the topic of the thesis presented and is structured in two chapters.

Chapter I presents generalities concerning biological systems for the degradation of leather productsand aspects of thenatural leather productscomposition. There is also detailed data on the description of the technologies of biological degradation of the natural fur remnants applied to the industrial.

Chapter II presents biochemical systems for the degradation of natural leather products as well as the description of the enzyme system involved in fur degradation.

The second part of the thesis consists of the original contributions made during the research.

The research theme pursued:

- Isolation, selection, characterization, identification and testing of micro-organisms with efficiency in accelerated degradation of waste from the leather industry;
- Experimentation of the biodegradability of the main components of the natural fur-tanned articles of chromium;
- Creating a fermented product that could be used as a bio fertilizer for grain and leguminous crops.

Chapter III contains details on the isolation and characterisation of the microbial strains capable of degrading the furs, materials and methods used in laboratory experiments on selection, identification and cultivation selected strains, as well as results and discussions on obtaining biological material and identifying it.

The isolation phase of microorganisms was carried out by composting fragments of leather and fur tanned with chromium from the Bucharest Leather Institute.

In order to isolate and identify bacteria with proteolytic activity, pieces of fur with chromium tanned skin, coming from different types of animals were left to composted in soil with pH 7.2 for 3 months.From the composted fur soil a sample was taken, from which 90 colonies were isolated in a first stage, applying common microbiological techniques.

Through the cultivation of isolated colonies, in casein-based environments It was found (what was to be expected, given the protein content of fur), that around isolated colonies was observed the occurrence of an opalescent halo indicating the protease activity of isolated microorganisms.

Based on the size of the halo observed around isolated colonies, after the development on agar environments (containing casein), three microbial strains called: DA7, DA10, DA13, which were then moved and stored on the agar jealousy at 4^{o}C.

The 3 microbial strains have been identified with the BIOLOG System – the Microbial Identification System, as bacterial strains. Following the tests, it was concluded that the three selected and identified strains are:

Brevundimonas diminuta (DA7),

Bacillus thuringiensis (DA10),

Bacillus cereus (DA13)

The selected and identified strains were cultivated in the submerged system, with a view to obtaining a partially purified enzymatic preparation, possibly used in the biochemical degradation of fur and leather waste from the textile industry; the enzyme product obtained has also been tested for colagenase, keratinolytic and lipolytic activity, given the cultivation and selection of strains on culture environments containing pieces of fur and natural leather.

Following the tests it was found that the best enzymatic activity was recorded by the DA10 strain, followed by the DA13 strain, to a pH of 7.0.

Chapter IV of the paper includes: experiments on obtaining an enzyme preparation for the biodegradation of waste from the leather industry, the materials and methods of analysis and control needed to determine the enzymatic activities and the results and discussions on optimizing the parameters of enzymatic degradation of fur products.

The thesis also established a flow to obtain an enzyme preparation, using in fermentation the three bacterial strains, selected and characterized, during the research performed in the doctoral period.

From the analysis of the results shown in Chapter 4, it can be observed that natural fur can be used as a source of carbon, with significant values of enzyme production in the case of isolated strains DA7, DA10, DA13 (fermentation media forproteolytic, colagenazic, keratinolytics and lipolytic activities).

Based on the results shown in this chapter, we can draw these conclusions:

The value of the concentration of natural fur shredded and added to the cultural medium in proportion of 0.6 g% can induce the biosynthesis of hydrolytic enzymes, capable of degrade the remnants of fur and suede with chromium, resulting from the textile industry.

The results of the experiments show that the highest production of protease is achieved in the case of cultivation of strains DA10 on the minimal medium, at a pH 7.0, equal to 0, 677U/ml/min

In the same chapter 4, the importanceof pH and temperature on enzyme production has been studied: collagen, keratinase, lipase, the following conclusions were reached:

The temperature influence on the collagen production for strain DA7 has completed the value of 0.380U/ml, for DA10, 0.401U/ml, and for DA13 the value was equal to 0.394/ml.

The temperature influences the production of keratinase, the best yield being at 35°C ,the following values may be observed 0.180 U/ml for strain DA7, 0.248 U/ml for strain DA10 and 0.223 U/ml registers the DA13 strain.

The temperature also has an influence on the production of lipase. The maximum production of lipase was obtained with DA10 strain at 35°C, after an incubation period of 120 hours, the value equal to 140 U/ml.

• From the measurements of the influence of pH on the activity of these enzymes, it can be ascertained, following the tests carried out, that the pH influences the production of lipase, with the values of 100 U/ml for strain DA7, 160 U/ml for DA10 and 160 U/ml/min for DA13 to pH neutral.

• The highest value for keratinases production is obtained with strain DA10, at a pH equal to 7.0 and 120 hours of incubation, i.e. 0.254 units/ml.

Biodegradability tests of natural fur were carried out, the organic carbon concentration of the culture medium was determined after adding pieces of fur and leather in the culture medium of the 3 strains.

• The organic carbon concentration is noticeably reduced, resulting from the microbial activity that used carbon from the fur pieces for enzymatic production, thus the organic carbon concentration decreased from 35.68% initially to 24.63% for strain DA7, 24.82% for the DA 10 strain and 25.75% for the DA13 strain, after the 120-hour incubation of the pieces of natural fur in the culture environment.

• The analysis of the obtained data shows an increase in the activity of the enzymatic complex proportional with the period of time during which the microbial strains were cultivated.

• The greatest keratinolytic productionactivity by isolated strains under the conditions of cultivation given was 0.223 U/ml versus the witness, produced by the DA10 strain.

The flow of conditioning and characterization of the enzyme preparation obtained with the three strains selected in the thesis:

The flow of obtaining and conditioning of the enzyme preparation was established, respectively:

• After the fermentation phase, the native solution separated from the biomass by centrifugation concentrated 10 times in a rotavapor, under vacuum, at a temperature of 35 degrees Celsius and then used as such in applications.

• After the concentration of hydrolysate obtained in the work, the best enzymatic activity has registered a strain DA10 as follows proteases 1.975 U/ml, colagenases 1.275 U/ml, keratinases 0.911 U/ml and lipases 80 U/ml.

The lyophilization process was used to increase the stability of the enzyme preparation of microbial origin,.

For obtaining a solid enzymatic preparation with the highest enzymatic activity, both methods were applied for the same sample the centrifuged fermentation fluid (for the removal of biomass) concentrated 10 times, at the Heidolph Rotavapor then the concentrate obtained was lyophilised.

The lyophilised enzyme preparation was characterized in terms of enzymatic activity after redissolution in 10 ml of distilled water, obtaining the following values:

Proteolytic activity (at pH = 7.0) = 19, 75U/ml

Colagenastic activity: 12.5 U/ml

Lipolicy activity: 800U/ml

Keratinolytic activity: 9U/ml

In chapter V of the thesis, test works of the liquid enzymatic concentrate obtained in the thesis were started, with a view to its recovery, as bio fertilising in organic farming.

The experiments consisted in testing the fermentation fluid obtained after the cultivation of the strains selected on cultural environments in which natural fur wastes and chromium-tanned leather were added, during the biosynthesis of enzymes there was a hydrolysis of shredded pieces of fur resulting in amino acids, fatty acids, lipids, keratin, proven raising up the amount of biomass in the environment and the determination of enzymatic activities.

After the biosynthesis was done filtering the fermentation environment, concentration 10 times at the rotavapor and then adding in different proportions to the germination soil of grain and leguminous seeds.

The addition of enzymatic preparation has benefical results in the growth and development of grasses and leguminous crops.

Of the three strains tested, *B. thuringiensis* was shown to be the most effective at in vitro testing as a bio fertiliser, adding in a concentration of 35% in the cultivation soil of grams and legumes.

The fermentation medium obtained by the cultivation of isolated strains having as a carbon source natural fur and leather provided from the Bucharest Leather Institute, has been processed by filtration and concentration obtaining a microbiologically concentrated product , liquid, used as a bio fertilizing addition in vegetable crops by *R. sativus, Triticum sp*. and *S. tuberosum*.

In the case of fertilization of *S. tuberosum* it's observed that the species also are an important factor of the development.

Chapter VI of the thesis comprises the conclusions and recommendations on research directions.

The research carried out in the thesis proved the possibility of obtaining enzymatic products capable of degrading the waste from natural fur as well as their recovery in other areas, such as obtaining bio fertilizing for plants.